1 Basic principles

1. What particles are magnetic? May a magnetic particle have a zero spin number? May a magnetic particle have a zero electric charge? Give examples of nuclei which are magnetic. What nuclei are found in biomacromolecules? Which of them are magnetic? Which of them can be routinely studied by NMR?

2. What is the relation between energy difference of stationary spin states and the external magnetic field. How is the frequency of precession related to the external magnetic field? What determines how many nuclei are in the individual states?

3. The precession frequency of protons in different chemical groups differ. Why? Give a relative size of these deviations (in orders of magnitude).

4. Which of the following interactions change the average precession frequency of nuclei in molecules dissolved in an isotropic solvents and which of them significantly contribute to relaxation: direct interaction of magnetic dipolar moments, shielding by electrons, interaction of magnetic dipolar moments mediated by electrons of chemical bonds connecting the interacting atoms.

5. Is the presence of a static homogeneous external magnetic field necessary to create the macroscopic magnetization? Is it sufficient to have the static homogeneous external magnetic field in order to create the macroscopic magnetization? Is it sufficient to have the static homogeneous external magnetic field in order to observe signal in an NMR spectrometer? If not, what else is needed?

6. Explain the following terms: ninety-degree pulse, carrier frequency, frequency offset.

7. What is the Fourier transform good for in NMR spectroscopy?

8. Explain the idea of a two dimensional experiment.

9. Describe evolution of chemical shift and scalar coupling during various echoes.

10. Describe INEPT.

2 NMR spectroscopy of nucleic acids

11. Using Fig.1, explain the terms purine base, pyrimidine base, nucleotide, and nucleoside. Which of the atoms are involved in forming hydrogen bonds in Watson-Crick and Hoogsteen base pairing?

12. In Fig. 1, show numbering of purine and pyrimidine bases and dihedral angles α, β, γ, δ, ϵ, ζ, and χ. Explain the concept of pseudorotation angle for describing sugar conformations.

13. Draw the structure formulas of Adenine, Cytosine, Guanine, Thymine and Uracil. Which of the hydrogen atoms are in the fast exchange regime with solvent water? How does the exchange affect the measured NMR spectra?

14. Which quick NMR experiment would you use for a newly prepared oligonucleotide sample to check whether it is suitable for further study by NMR? Which of the following solvents would you use to prepare the sample and why? (H_2O pure, D_2O pure, mixture 90% H_2O and 10% D_2O).

15. Explain the syn- and anti conformations around the glycosidic bond. How would you use NMR spectroscopy to distinguish between these two conformations?

16. What is sugar puckering? Which sugar conformations are prevalent in DNA and RNA? How can you study the sugar conformation by NMR spectroscopy?

17. Is the spectrum in Fig. 2 COSY or NOESY spectrum? What kind of interaction gives rise to the cross-peaks in this type of spectra? Is the spectrum in Fig. 2 one of a DNA or RNA? How many nucleosides of each kind does the molecule include provided the sample is a symmetric duplex and all the bases form Watson-Crick base pairs?

18. Using Fig. 3–5, explain the principle of sequential assignment in nucleic acids using NOESY spectra.

19. Explain the difference between the assignment of nucleic acids using through-space (NOESY) and through-bond interactions. Which of the methods is more reliable and why? What kind of nucleic acid sample do you need for the through-bond triple-resonance experiments to be applicable?

20. Name the most important heteronuclear experiments used for the assignment of nucleic acids and explain their use.

3 NMR relaxation

21. Describe two most important physical mechanisms of NMR relaxation (for spin-1/2 nuclei). Describe their contributions to the R_1 and R_2 relaxation rates.

22. Describe the terms "correlation function" and "correlation time" for a rigid spherical molecule in the absence of chemical/conformational changes modifying the chemical shift tensor. Use the loss of coherence as an example.

23. How is NMR relaxation related to molecular motions? Describe the term "spectral density function". Describe the effect of fluctuating fields on the loss of coherence and on the return of the total magnetization to its equilibrium value.

24. What is the effect of slow (slower than the correlation time of the overall tumbling) chemical/conformational changes modifying the chemical shift tensor on NMR relaxation? How are the relaxation rates R_1 and R_2 influenced?

25. What is the effect of internal motions of biomolecules on relaxation of their NMR signals? Use an example of relaxation of ^{15}N in amide groups in the protein backbone and assume that the internal motions and the overall tumbling are independent.

26. Describe the principles of measuring relaxation rates in biomolecules. What relaxation parameters are usually measured in practice?

27. What methods can be used to interpret NMR relaxation parameters in the terms of intramolecular motions? Explain the basic principles.

28. Describe the procedure for the model-free analysis of relaxation data.

29. On what assumptions is the model-free analysis based?

30. Describe the procedure of the spectral density mapping. Under what conditions is the reduced spectral density mapping applicable?

Extra questions for those who did not attend/pass the practical course C6775:

4 Assignment of protein NMR spectra

1. Draw examples of individual ^{1}H, ^{15}N, and ^{13}C spin systems in a protein chain.

2. How many peaks should appear in a well-resolved ^{1}H-^{15}N HSQC spectrum of a peptide MTHLNKWPEQ?

3. How many peaks should appear in a well-resolved ^{1}H-^{15}N HSQC spectrum of a peptide MYPCTGQNLE?

4. How many peaks should appear in a well-resolved ^{1}H-^{15}N HSQC spectrum of a peptide MIGPWLKNVD?

5. With the help of the table of typical carbon chemical shifts, assign the following chemical shifts (obtained from an HNCACB experiment) to alanine, tyrosine, tryptophan, glycine, and threonine: (62.3 ppm and 68.4 ppm), (50.3 ppm and 18.4 ppm), (56.3 ppm and 39.4 ppm), (42.3 ppm), and (60.3 ppm and 29.4 ppm).

6. With the help of the table of typical carbon chemical shifts, assign the following chemical shifts (obtained from an HNCACB experiment) to alanine, isoleucine, histidine, glycine, and serine: (62.3 ppm and 38.4 ppm), (53.3 ppm and 28.4 ppm), (50.3 ppm and 19.4 ppm), (44.3 ppm), and (60.3 ppm and 57.4 ppm).

7. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.23/62.1, 2.01/31.9, 0.63/20.4, and 0.81/21.9.

8. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.93/59.1, 4.35/62.9, and 4.13/62.9.

9. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.53/62.1, 5.35/69.9, and 1.23/22.9.

10. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.23/60.1, 3.35/41.9, 3.25/41.9, 2.00/30.7, 1.85/30.7, 1.92/28.4 and 1.63/28.4.

5 NMR structure determination

11. Describe difference between chemical conformation and configuration of biomacromolecules, their relative importance in the structure determination of small and large molecules, role of torsion angles.

12. Describe how the three-bond scalar coupling constant between amide and alpha proton reflect secondary structure of the protein.

13. Briefly explain at least one method of the three-bond scalar coupling measurement.

14. What inter-proton distances serve as good indicators of β-sheets and helical structures. Is it possible to distinguish α and 3_{10} helix based on NOE cross-peaks (if so, how?)?

15. How can one predict secondary structure just from chemical shift values?

16. What structural information can be obtained from residual dipolar couplings and how is the residual dipolar coupling measurement done in practice?

17. It is not possible to calculate three-dimensional structure from the measured distances by simply solving the trigonometric equations (distance geometry). Explain why.

18. Compare advantages and disadvantages of using different structure-related types of NMR data (NOE, scalar couplings, dipolar couplings) in the structure determination.

19. Briefly describe the principles of molecular dynamics simulations. Why do we use experimental data when computational methods are available.

20. Explain how NMR data are introduced into the structure calculations using molecular dynamics.

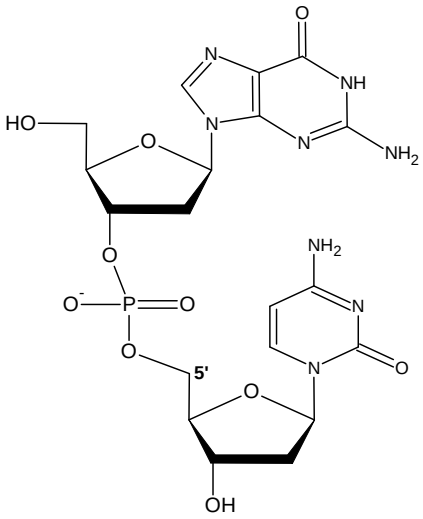


Figure 1:

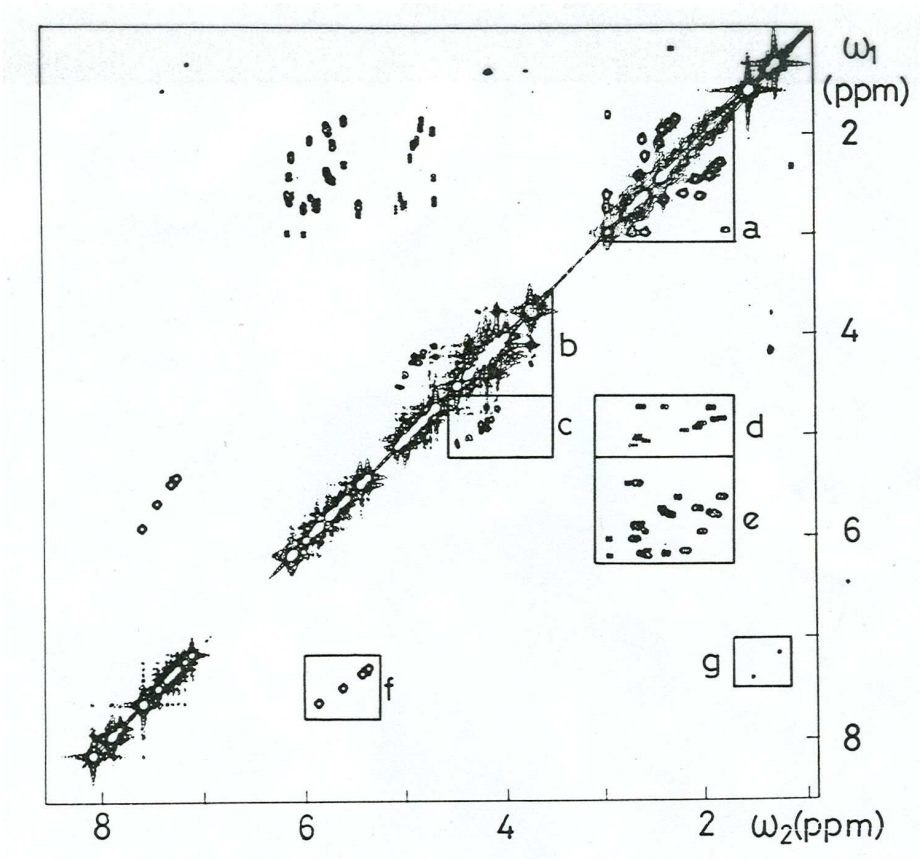
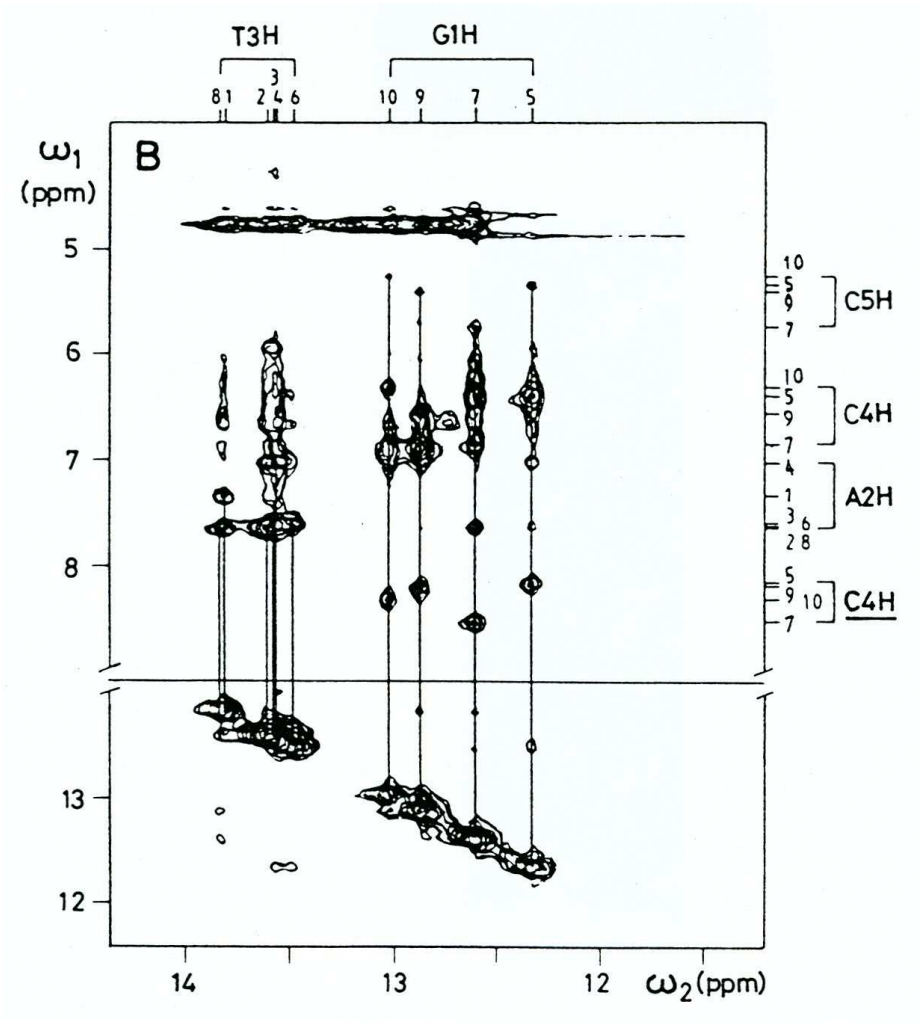


Figure 2: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ω1
(ppm)
5
6
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ω2(ppm)
C5H
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A2H
C4H

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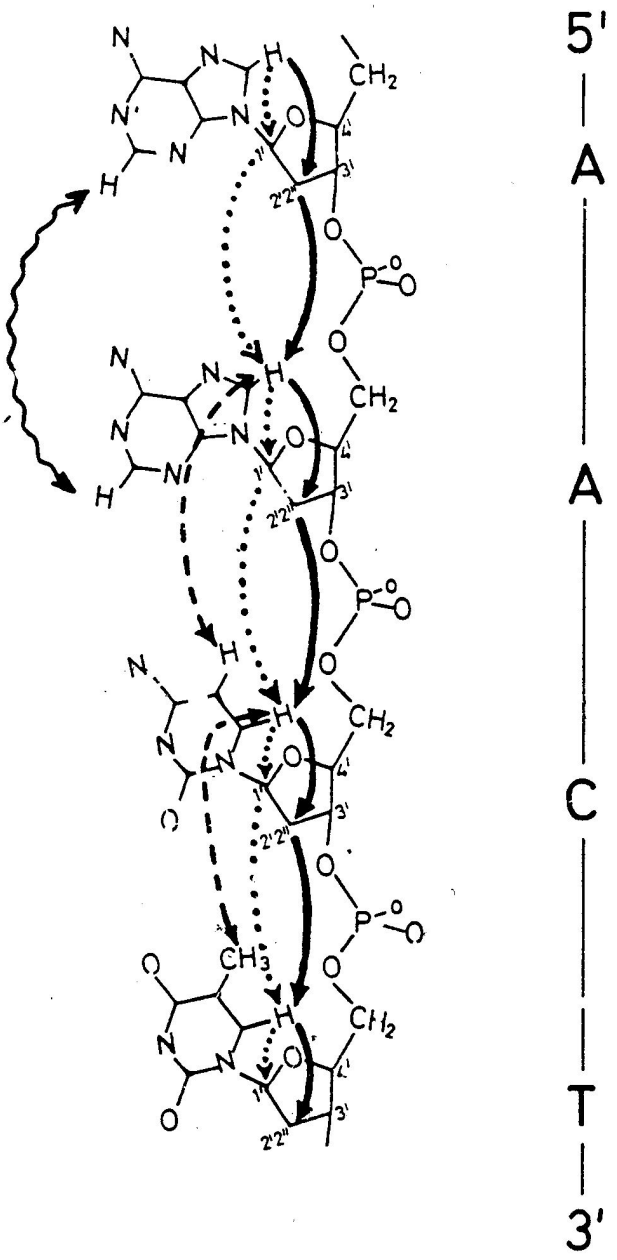

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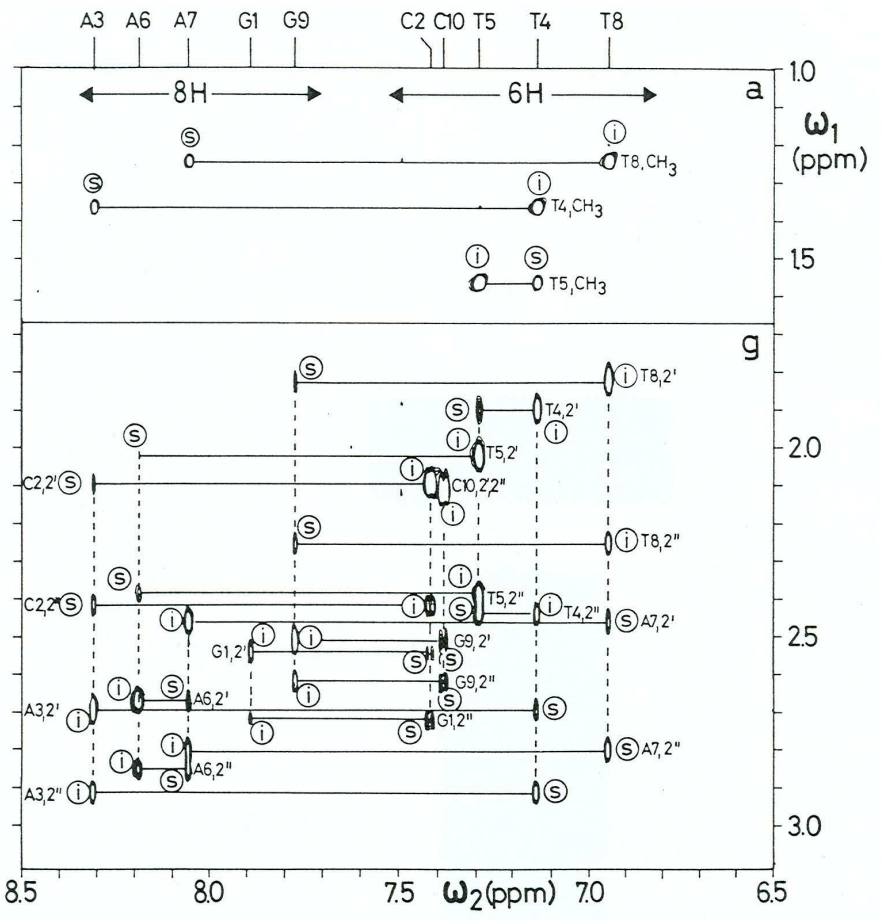
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ω1 (ppm)
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ω2(ppm)
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Figure 5: